46. (Amended) The method of claim 43, wherein said hard base chelator is DTPA, NOTA, DOTA or TETA.

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47. (Twice Amended) The method of claim 33, wherein said at least one other arm specifically binds a tyrosyl-lysine dipeptide.

48. (Twice Amended) The method of claim 33, wherein said at least one other arm specifically binds Tyr-Lys(DTPA)-NH_2, or Lys(DTPA)-Tyr-Lys(DTPA)-NH_2..

REMARKS

Claims 31, 33, 34, 37-48, and 50 are pending and have been rejected. Claims 31 and 50 have been cancelled, without prejudice or disclaimer to the filing of one or more divisional applications to pursue subject matter recited therein. Claims 33 and 34 have been amended to replace Fab' by Fab. No new issue is raised by this amendment, as it merely returns the scope of the fragment in claims 33 and 34 to the scope that was pending prior to the amendment of December 3, 2001. At that time, there was no prior art applied against the method claims, but Fab was changed to Fab' in the method claims to be consistent with changes to the product claims 31 and 50. The product claims now are being canceled, and the scope of the fragment in the method claims is being changed back to the scope in the original claims. Entry of these amendments to the method claims, and a continued indication of their allowability, is respectfully requested.

Claims 31, 37-48, and 50 are rejected under the second paragraph of Section 112 as being indefinite. Claims 31 and 50 have been canceled and claims 37-48 have been amended to replace "construct" with "method." Claims 37 and 38 have also been amended to recite that "at least one other arm that specifically binds a targetable conjugate is a humanized Fab fragment." Reconsideration and withdrawal of the rejection under the second paragraph of Section 112 is respectfully requested.

Applicants note with appreciation that the previous rejection under Sections 101 and 103 have been withdrawn.

Claims 31 and 50 are rejected under the first paragraph of Section 112, and under Sections 102(b) and (e) based Bosslet *et al.* (U.S. 5,591,828). Claims 31 and 50 have been canceled.

In view of the foregoing remarks, it is believed that all claims are in condition for allowance. Reconsideration of all rejections and a notice of allowance are respectfully requested. Should there be any questions regarding this application, the examiner is invited to contact the undersigned attorney at the phone number listed below.

Respectfully submitted,

James E. Pauls Reg. No. 45,651 for

March 10, 2003
Date

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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

Version with markings to show changes made in the claims

Please cancel claims 31 and 50 and amend the remaining claims as follows:

31. Canceled

33. (Amended) A method of preparing a bi-specific [Fab'-scFv] Fab-scFv fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, comprising:

(1) (A) introducing into a mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) co-introducing into said mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in (A) and which when associated with said Fd fragment forms a [Fab'] Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region

functional in said mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions;

(C) growing said cell; and

(D) isolating said bi-specific [Fab'-scFV] Fab-scFv fusion protein, or

(2) (A) introducing into a first mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first mammalian host cell, a translational initiation regulatory region functional in said first mammalian host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said first mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) introducing into a second mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second mammalian host cell a light-chain antibody fragment which is complementary to said Fd fragment in (2)(A) and which when associated with said Fd fragment forms a [Fab'] Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second mammalian host cell, a translational initiation regulatory region functional in said second host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said second mammalian host cell, wherein expression of said light-chain antibody fragment is under the control of said regulatory regions;

(C) growing said first and second mammalian host cells;

(D) optionally isolating said bi-specific fusion protein fragment and said light-chain antibody fragment;

(E) combining said fragments to produce a [Fab'-scFV] Fab-scFv bi-specific fusion protein; and

(F) isolating said bi-specific fusion protein.

34. (Amended) A method of preparing a bi-specific [Fab'-scFV] Fab-scFv fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes, comprising:

(1) (A) introducing into a mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said mammalian host cell, a translational initiation regulatory region functional in said mammalian host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) co-introducing into said mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said mammalian host cell a Fd fragment which is complementary to said light-chain antibody fragment in (A) and which when associated with said light-chain antibody fragment forms a [Fab'] Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region

functional in said mammalian host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said mammalian host cell, wherein said expression of Fd fragment is under the control of said regulatory regions;

(C) growing said cell; and

(D) isolating said bi-specific [Fab'-scFV] Fab-scFv fusion protein, or

(2) (A) introducing into a first mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first mammalian host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first mammalian host cell, a translational initiation regulatory region functional in said first mammalian host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said first mammalian host cell, wherein expression of said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) introducing into a second mammalian host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second mammalian host cell a Fd fragment which is complementary to said light-chain antibody fragment in (2)(A) and which when associated with said light-chain antibody fragment forms a [Fab'] Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second mammalian host cell, a translational initiation regulatory region functional in said second mammalian host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said second mammalian host cell,

wherein expression of said Fd fragment is under the control of said regulatory regions;

(C) growing said first and second mammalian host cells;

(D) optionally isolating said bi-specific fusion protein fragment and said Fd fragment; and

(E) combining said fragments to produce a bi-specific [Fab'-scFV] Fab-scFv fusion protein; and

(F) isolating said bi-specific fusion protein.

37. (Twice Amended) The [construct] method of claim 33, wherein said at least one arm that specifically binds a targeted tissue is a humanized [antibody or a] Fab fragment [of a humanized antibody].

38. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm that specifically binds a targetable conjugate is a humanized [antibody or a] Fab fragment [of a humanized antibody].

39. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a peptide.

40. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a carbohydrate.

41. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a hapten.

42. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm specifically binds said epitope of said targetable conjugate, and said epitope comprises a chelator or a metal-chelate complex.

43. (Amended) The [construct] method of claim 42, wherein said chelator is a hard base chelator for a hard acid cation.

44. (Amended) The [construct] method of claim 42, wherein said chelator is a soft base chelator for a soft acid cation.

45. (Amended) The [construct] method of claim 43, wherein said chelator is a hard base chelator that comprises carboxylate and amine groups.

46. (Amended) The [construct] method of claim 43, wherein said hard base chelator is DTPA, NOTA, DOTA or TETA.

47. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm specifically binds a tyrosyl-lysine dipeptide.

48. (Twice Amended) The [construct] method of claim 33, wherein said at least one other arm specifically binds Tyr-Lys(DTPA)-NH_2, or Lys(DTPA)-Tyr-Lys(DTPA)-NH_2..

50. Canceled